Author's response to reviews

Title: Lemon juice has protective activity in a rat urolithiasis model

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Version: 3 Date: 5 September 2007

Author's response to reviews: see over
4th September 2007

Dear Editor,

Following your instructions, we are sending you the revised manuscript of the paper entitled “Lemon juice has protective activity in a rat urolithiasis model”. We also include a cover letter with the response to the reviewers.

Being at your disposal, yours faithfully,

Prof. Dr. F. Grases
Cover letter giving response to the Reviewer’s comments.

**Reviewer P. Varalakshmi**

We thank the comments of the Reviewer that allow to a clear improve of the paper.

With reference to the urinary parameters, it must be considered that the lithogenic effects of ethylen glycol (EG) must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by this substance. For this reason, the presented studies were focused to evaluate the effects on renal papillary tissue through histological studies and the protective effects caused by the consumption of lemon juice. Previous studies evaluated the effects of citrate on renal lithiasis induced by EG [43-44]. Nevertheless, to attain an increase in citrate excretion it is necessary to induce metabolic acidosis in rats and to achieve this condition it is necessary to increase the doses of EG to 2%. In such case, urinary pH of EG treated rats was clearly inferior to urinary pH of control group, the treatment with high doses of potassium citrate significantly increased the urinary pH and, as a consequence, the urinary citrate excretion notably rose. Nevertheless, EG doses of 0.75% practically did not change the urinary pH value when compared with control group [36, 44] and consequently the administration of citrate did not cause important changes in urinary citrate excretion [45]. On the other hand, previous studies on EG supplied to rats demonstrated that administration of herbal extracts with prophylactic potential apparently did not affect to urinary biochemistry [12-13, 34-35], and for all these reasons no urinary biochemical studies were included in the present paper.

All these aspects are now included and discussed in the paper: page 8, lines 26-35; page 9, lines 1-2.

As it is commented by other Reviewers, the toxicity induced by ethylene glycol is the cause of the body weight loss, that is always observed when this substance is used as an agent to induce calcium oxalate precipitation in rats.

Note: References numbers correspond to paper reference numbers.

**Reviewer M. Boim**

We thank the comments of the Reviewer that permitted us to clarify and improve the paper.

With reference to the use of ethylen glycol to induce lithiasis in a rat model, now in the Introduction section this aspect is extensively commented (page 3, lines 8-27).

Problems associated to images and table were detected and now they are better presented to avoid confusion.
Following the reviewer observation, the statistical analysis is repeated using variance analysis (ANOVA).

6 µl of lemon juice was the chosen dose because, considering a 6% of citric acid content in lemon juice and the weight of approximately 280 g for the used rats, this would correspond to a dose of 0.1 g of citric acid for rat and day, that is similar to the equivalent doses used in humans.

The reasons why urinary parameters were not included are now explained in the Discussion section (page 8, lines 26-35; page 9, lines 1-2).